Page 26, line 7, delete "target" and insert --population of screening cells--;

line 13, after "marker" insert --recognized by the selected mutant--.

In the claims:

Please amend the claims as follows:

a7

1. (amended) A method for [identifying] making a cytotoxic mutant protein[s] or pool of proteins of a cytotoxic wild type protein said mutant protein or pool of proteins having a different receptor-binding specificity than the wild type protein, [said cytotoxic mutant proteins capable of binding to a target cell which the wild type protein does not selecting bind to,] comprising:

(A) selecting a heteromeric protein toxin having a toxic domain or subunit and a binding domain or subunit;

(B) incorporating mutations into DNA encoding the binding domain or subunit of the heteromeric protein toxin to produce a plurality of variant forms of the heteromeric protein toxin;

(C) generating a library of microorganism clones producing variant forms of the heteromeric protein toxin[s by incorporating mutations into the binding subunit DNA of the heteromeric protein toxin]; [and]

[(C)](D) screening the variant forms of the heteromeric protein toxin of said library against [said target] a population of screening cells which are substantially insensitive to the cytotoxic wild type protein by isolating clones or pools of clones producing said variant forms of the heteromeric protein toxin[s], treating preparations of said [target] population of screening cells with [said] variant forms of the heteromeric protein toxin[s] produced by the isolated clones or pools of clones, and selecting a cytotoxic mutant protein or pool of cytotoxic mutant proteins that inhibits or kills said [target] population of screening cells; and

(E) making additional copies of the selected cytotoxic mutant protein or pool of proteins.

a7 contd

2. (amended) The method of claim 1, wherein the [target] cells in the population of screening cells are [is] eukaryotic.

Claim 5, line 1, after "binding" insert --domain or--.

a8

6. (amended) The method of claim 1, wherein said mutation is incorporated into said binding domain or subunit by use of a combinatorial cassette method comprising:

(A) preparing synthetic mutant oliognucleotides capable of annealing with a corresponding wild type oligonucleotide from said binding domain or subunit;

(B) annealing said synthetic oligonucleotide from said binding domain or subunit to an overlapping wild type oligonucleotide to form a double stranded sequence;

(C) creating a combinatorial cassette by mutually primed synthesis of said [oligo] double stranded sequence; and

(D) incorporated said cassette into a vector containing a gene for said toxin.

Claim 7, line 2, after "binding" insert --domain or--.

Claim 8, line 2, delete "comprising" and insert --consisting of--.

Claim 9, line 2, delete "comprising" and insert --consisting of--.

Claim 11, line 1, delete "random".

Claim 12, line 1, delete "random".

a9

13. (amended) The method as claimed in claim 2 wherein [said target cell is] the cells in the population of screening cells are tumour cells.

14. (amended) The method of claim 13 wherein [said target cell is a] the tumour cells are breast cancer cells.

a 16

16. (amended) The method as claimed in claim 1 wherein said binding domain or subunit is derived from the B-subunit template of either Shiga toxin or Shiga-like toxins, or homologous counterparts from *E. coli* heat labile enterotoxins, cholera toxin, pertussis toxin or the receptor binding domain of ricin.

17. (amended) A method of killing or inhibiting a target cell comprising treating said target cell with [said] a cytotoxic mutant protein or pool of proteins made in accordance with the method of claim 1, wherein said target cell expresses a receptor to which the cytotoxic mutant protein or pool of proteins specifically binds.

18. (amended) A method for identifying therapeutic proteins having binding specificity for a target cell, comprising:

(A) [identifying] making a cytotoxic mutant protein[s] or pool of proteins by the method as claimed in claim 1; and

(B) screening said cytotoxic mutant protein[s] or pool of proteins against said target cells and against non-target cells by treating a preparation of target and a preparation of non-target cells with said cytotoxic mutant protein[s] or pool of proteins, and selecting a therapeutic protein or pool of therapeutic proteins that are effective to inhibit or kill said target cells and that are less effective at inhibiting or killing said non-target cells than at inhibiting or killing said target cells.

Claim 20, line 1, change "claim 19" to --claim 28--.

Claim 24, line 3, change "claim 22" to --claim 32--.

Claim 25, line 3, change "claim 23" to --claim 33--.

Please cancel claims 19, 21, 22, 23 and 26 add new claims 27-33 as follows:

--27. A method for constructing a diagnostic probe for detecting the presence of a cell surface marker comprising:

(A) selecting a cytotoxic mutant protein that specifically binds to the cell surface marker by the method as claimed in claim 1; and

(B) preparing a diagnostic probe by labeling the selected cytotoxic mutant protein in a manner which maintains the ability of the binding domain or subunit of the selected cytotoxic mutant protein to specifically bind to the cell surface marker.

28. The method of claim 27, wherein the diagnostic probe is prepared by a method comprising:

(i) preparing a diagnostic DNA sequence comprising a marker DNA encoding a detectable marker and a binding domain or subunit DNA sequence encoding the binding domain or subunit of the selected cytotoxic mutant protein; and

(ii) expressing the diagnostic DNA sequence to generate a diagnostic probe.

29. The method of claim 27, further comprising the step of modifying the cytotoxic mutant protein or pool of proteins by dissociation or inactivation of the toxic domain or subunit of the cytotoxic mutant protein.

30. A composition comprising a cytotoxic heteromeric protein comprising a cytotoxic subunit and a binding subunit, said cytotoxic heteromeric protein being derived by mutation of Shiga toxin or a Shiga-like toxin at one or more locations within the binding subunit of the Shiga toxin or Shiga-like toxin, said locations being located in loops harbouring residues involved in creating a receptor binding cleft for CD77, wherein as a result of the mutation the cytotoxic protein does not bind to CD77 but does bind with cytotoxic effect to one or more other receptors.

a 11 cont

31. The composition of claim 30, wherein the Shiga-like toxin is Shiga-like toxin 1 and wherein the mutations are located in residues 15-19, 30-33 or 58-64 of the binding subunit.

32. A method for making a targeted medicament for delivery to a target cell having a cell surface marker, said targeted medicament comprising a binding portion and a medicament portion comprising the step of:

(A) identifying a binding subunit which binds to the cell surface marker by a process comprising the steps of

(i) selecting a heteromeric protein toxin having a toxic domain or subunit and a binding domain or subunit;

(ii) incorporating mutations into DNA encoding the binding domain or subunit of the heteromeric protein toxin to produce a plurality of variant forms of the heteromeric protein toxin;

(iii) generating a library of microorganism clones producing variant forms of the heteromeric protein toxin;

(iv) screening the variant forms of the heteromeric protein toxin of said library against a population of screening cells which express the cell surface marker and which are substantially insensitive to the cytotoxic wild type protein by isolating clones or pools of clones producing said variant forms of the heteromeric protein toxin, treating preparations of said population of screening cells with variant forms of the heteromeric protein toxin produced by the isolated clones or pools of clones, and selecting a cytotoxic mutant protein or pool of cytotoxic mutant proteins that inhibits or kills said population of screening cells; and

(v) determining the sequence of the binding domain or subunit of the selected cytotoxic mutant protein for use as the binding portion of the targeted medicament; and

(B) combining the binding portion with the medicament portion.

33. The method of claim 32, wherein the binding portion and the medicament portion are combined by preparing a medicament DNA sequence comprising a medicinal DNA encoding a medicinal polypeptide for use as the medicament portion, and a binding domain or subunit DNA sequence encoding the binding portion, further comprising the step of expressing the medicament DNA sequence. --

REMARKS

The specification has been amended to reference the parent PCT application of which this is a section 371 national phase filing, and to correct a typographical error in the date of the Canadian priority application.

During prosecution before the International Preliminary Examination Authority (IPEA/EP), the Examiner asserted that some of the claims then pending lacked novelty in view of two references, Jackson et al., *J. Bacteriol.* 172: 653-658 (1990) and Perera et al., *J. Bacteriol.* 173: 1151-1160 (1991). After review of these references and the previously presented claims, it appeared that the claims needed to be amended to more clearly distinguish from these disclosures which are in fact quite different from the present invention. That is done in this amendment.

In particular, both the Jackson et al., and the Perera et al. references relate to studies in which the mutations were made to assess which amino acids in the binding subunit of Shiga toxin (ShT) or Shiga-like toxin (SLT) were important for activity of the toxin. Thus, the mutants which were developed were tested to determine their activity against cells which were known to be sensitive to wild type ShT and SLT. In contrast, as more clearly set forth in amended claim 1, the present invention makes use of a population of screening cells which are substantially insensitive to the wild-type toxin. Such a screening procedure could not yield the results described in Jackson et al. and Perera et al., and is thus neither anticipated nor suggested by these disclosures.

The amendment to claim 1 is in the nature of clarification only and does not introduce new matter. For example, the statement that the mutant has a different receptor-